UNIVERSITI PUTRA MALAYSIA

REDUCTIVE ALKYLATION OF *CANDIDA RUGOSA* LIPASE : STRUCTURAL APPROACHES

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by

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

September 2004

Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Doctor of Philosophy

REDUCTIVE ALKYLATION OF *CANDIDA RUGOSA* LIPASE: STRUCTURAL APPROACHES

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September 2004

Chairman : Professor Abu Bakar Salleh, Ph.D.

Faculty : Biotechnology and Biomolecular Sciences

The properties of alkylated lipase are successfully explored through experimental and molecular modelling methods. Alkylation was done using aldehydes with different degree of modification to represent different levels of hydrophobicity which is important for enzymes to work in nonaqueous environment.

Far ultraviolet circular dichroism (CD) spectroscopy of the lipase in aqueous solvent shows that increasing the degree of modification from 49% to 86% resulted in loss in secondary structure which is attributed to the enzyme unfolding. The secondary structure elements of the CD spectra of native and modified lipase were analysed using the CDPro software and the K2D program. Both methods yield the same results in that the ratio of α-helical

structure is lost. This result explains why alkylated lipases have much lower activity in an aqueous environment.

Molecular modelling simulations were performed to study the structural and dynamical changes of the lipase upon different levels of modification. Simulations were run for 1 ns (300 K) with five different initial velocities to obtain better conformational sampling. Two solvent systems were used: TIP3P water model and carbon tetrachloride (CCl_4) solvent model in periodic boundary condition (PBC). Generally, lipases simulated in water are less deviated in term of root mean square deviations (rmsd) compared to lipases simulated in CCl_4.

Lid movements are essential for lipase function, both in water and water-lipid environments. Analyses of lid dynamics were done using time-correlation function and second-order Legendre polynomial function. Lipase in water and CCl_4 shows different properties of dynamics. Without alkylation, the time correlation function of lipase in water shows one slow exponential decay with a correlation time of τ = 92.8 ps. In contrast, for simulations in CCl_4 the lid has a more complex dynamics. Exponential fit of open CRL in CCl_4 results in two different τ values: a fast motion τ_1 = 5.6 ps and a slow motion τ_2 = 163.8 ps.

Upon alkylation, different levels of modification show different properties of lid motions. In CCl_4, lid region is highly stabilised upon 95% alkylation with slow motion mode of τ_1 = 4.1 ps and τ_2 = 577.8 ps. Slow motion effect of lid region is also observed at 63% with τ_1 = 2.9 ps and τ_2 = 209.2 ps and 43% modification with τ_1 = 3.4 ps and τ_2 = 117.9 ps. In water, 43% and 95% modification show similar motion with unmodified lipase, with one slow exponential decay of τ = 142.8 ps and 133.6 ps, respectively. However, 63% modification shows more complex dynamics with different τ values which mimics the dynamics properties in CCl_4.

A novel lid-locking mechanism which stabilises the opening form of lid region has been observed during simulations of unmodified CRL in CCl_4, *i.e.* a salt bridge between Lys85 and Asp284. This salt bridge is highly stabilised on unmodified lipase with a distance of 3.3 Å compared with lipase simulated in water with a distance of 15.25 Å. Alkylation at 43% causes the salt bridge to be deformed in CCl_4 with a distance of 6.03 Å; however, 63% modification stabilises the salt bridge with a distance of 3.88 and 95% modification shows the most stabilising effect with a distance of 3.19 Å.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENGALKILAN REDUKSI LIPASE *CANDIDA RUGOSA*: PENDEKATAN STRUKTUR

Oleh

BIMO ARIO TEJO

September 2004

Pengerusi : Profesor Abu Bakar Salleh, Ph.D

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Sifat-sifat lipase teralkil telah berjaya dikaji dengan kaedah uji kaji dan dinamik molekul. Pengalkilan dilakukan dengan aldehida pada beberapa peringkat pengubahsuaian yang berbeza untuk menggambarkan perbezaan hidrofobisiti yang penting bagi enzim untuk bekerja dalam persekitaran tanpa air.

Spektroskop "circular dichroism" sinar ultraungu jauh menunjukkan bahawa kenaikan peringkat pengubahsuaian dari 43% kepada 86% menyebabkan kehilangan struktur kedua yang berhubung kait dengan struktur enzim yang lebih terbuka. Unsur-unsur struktur kedua spektrum CD bagi lipase semula jadi dan terubah suai telah dianalisis menggunakan program CDPro dan K2D. Kedua kaedah itu menghasilkan keputusan yang sama bahawa nisbah struktur α-helix telah menurun. Keputusan ini memberi

penjelasan mengapa enzim lipase terubah suai memiliki aktiviti rendah di persekitaran yang mengandungi air.

Penyerupaan model dinamik molekul telah dijalankan untuk mengkaji struktur dan dinamik enzim lipase pada peringkat pengubahsuaian yang berbeza. Penyerupaan model telah dijalankan selama 1 ns (300 K) dengan lima kelajuan awal yang berbeza untuk mendapatkan contoh-contoh struktur molekul yang lebih baik. Dua sistem pelarut telah digunakan: model molekul air TIP3P dan model pelarut karbon tetraklorida (CCl_4) dalam keadaan berkala bersempadan (PBC). Secara amnya, lipase yang menjalani penyerupaan dalam pelarut air adalah kurang menyimpang dari segi "root mean square deviation" (rmsd) berbanding dengan lipase yang menjalani penyerupaan dalam pelarut CCl_4.

Pergerakan kawasan penutup adalah penting untuk fungsi lipase samada di dalam air atau dalam persekitaran yang mengandungi air-lipid. Analisis dinamik kawasan penutup telah dilakukan dengan menggunakan fungsi pertalian masa dan fungsi polinomial Legendre urutan kedua. Lipase dalam air dan CCl_4 menunjukkan sifat-sifat dinamik yang berbeza. Tanpa pengalkilan, fungsi pertalian masa lipase dalam air menunjukkan satu pereputan eksponen yang lambat dengan pertalian masa τ = 92.8 ps. Sebaliknya, kawasan penutup menunjukkan dinamik yang lebih rumit ketika

dilakukan penyerupaan dalam pelarut CCl_4. Kepadanan eksponen CRL terbuka dalam pelarut CCl_4 menunjukkan dua pertalian masa yang berbeza: satu pergerakan cepat τ_1 = 5.6 ps dan satu pergerakan lambat τ_2 = 163.8 ps.

Dengan pengalkilan, beberapa peringkat ubah suai yang berbeza menunjukkan sifat-sifat dinamik kawasan penutup yang berbeza. Dalam CCl_4, kawasan penutup sangat stabil pada pengalkilan 95% dengan pergerakan lambat τ_1 = 4.1 ps dan τ_2 = 577.8 ps. Pergerakan lambat kawasan penutup juga dilihat pada pengalkilan 63% dengan τ_1 = 2.9 ps dan τ_2 = 209.2 ps dan pada pengalkilan 43% dengan τ_1 = 3.4 ps dan τ_2 = 117.9 ps. Dalam air, pengubahsuaian 43% dan 95% menunjukkan pergerakan yang serupa untuk lipase semula jadi, dengan satu pertalian masa yang lambat, masing-masing 142.8 ps dan 133.6 ps. Tetapi, pengubahsuaian 63% menunjukkan dinamik yang lebih rumit dengan dua nilai τ berbeza yang menyerupai sifat-sifat dinamiknya dalam CCl_4.

Mekanisma baru tentang penguncian kawasan penutup yang menstabilkan bentuk terbuka lipase telah dilihat dalam penyerupaan lipase semula jadi dalam CCl_4, iaitu satu jambatan garam Lys85 dengan Asp284. Jambatan garam ini sangat stabil pada lipase semula jadi dalam CCl_4 dengan jarak 3.3 Å berbanding lipase dalam air dengan jarak 15.25 Å. Pengalkilan 43% menyebabkan jambatan garam menjadi cacat dalam CCl_4 dengan jarak 6.03

Å; tetapi pengubahsuaian 63% menstabilkan jambatan garam tersebut dengan jarak 3.88 Å dan pengubahsuaian 95% menunjukkan kesan penstabilan terbaik dengan jarak 3.19 Å.

ACKNOWLEDGEMENTS

I must express heartfelt thanks to my supervisor, Professor Abu Bakar Salleh for scientific challenge he has given me. His encouragement has been a source of my enthusiasm to explore this novel research area in the country. I have learnt so many things from him, not only as a researcher, but also about life. Thanks, Prof!

I am also grateful to my co-supervisors: Professor Mahiran Basri, Associate Professor Dr. Raja Noor Zaliha Raja Abd. Rahman, and Associate Professor Dr. Basyaruddin Abd. Rahman for their invaluable guidance, comments, and constant support during the period of this study. My deep appreciation is also extended to Associate Professor Dr. Che Nyonya Abd. Razak who was my co-supervisor; I felt such a loss when she decided to retire in the middle of my study.

For the scientists around the world who helped me to complete this challenging job, I would like to thank all of them. I have met them either physically and virtually through our communications by e-mails. Some of them came to Malaysia: Professor Teruna Siahaan (Kansas University), Dr. Mitsuru Haruki (Osaka University), and Dr. Farid Khan (Cambridge University). Thank you for coming! Some of them have assisted me through

e-mails: Professor Romas Kazlauskas (McGill University), Professor Karl Hult (Royal Swedish Institute of Technology), and Professor Sergio Emanuel Galembeck (Universidade de Sao Paulo). Nice to talk to you, guys!

I also would like to thank Dr. Juergen Pleiss for accepting and allowing me to work in his laboratory in Stuttgart. I have experienced working with one of the most advanced computing facilities in Germany during my stay there. Beside scientific matters, I have also enjoyed a good friendship with my German colleagues: Dr. Erik Henke, Dr. Sandra Barth, Dr. Peter Oelschlaeger, Markus Fischer, Florian Barth, Fabian Boes, and Alexander Steudle. *Danke schön!*

I also would like to thank Professor Rahmah Mohamed and Firdaus Raih for allowing me to work in their laboratory in Bangi. Special thanks to Associate Professor Dr. Sharifuddin Zain for introducing me to computational chemistry and a friendship we have built.

I must also express my gratitude to all my lab mates in Malaysia, especially Kak Shidah, Thanges, Kak Su, Laith, Leow, Ain, Ina, Rofandi, Ferrol, Aiman, Shah, Mohammad, Daim, Ghani, Shukuri, and Azreen for their sincere friendship, support, and their help. It was nice to spend my life in the lab with them.

I would like to acknowledge the Malaysian Government for financial support through the GRA scholarship and to German Ministry of Education and Research for financial support during my research visits to Stuttgart.

I must express my heartfelt thank to Azira for her patience and moral support I much needed during writing process of this thesis. I appreciate her understanding for many evenings and weekends that we could not spend them together while I was working. As a scientist with swingy mood, I am indeed very much lucky to have someone with extra patience and understanding like her.

Finally, my deepest appreciation goes to my family: Bapak, Ibu, Mas Koko, Mbak Devita, Mbak Ita, Mas Kukuh, and Dik Galuh for being so supporting, for their love and encouragement. Thank you, Bapak…thank you, Ibu…I dedicate this work to both of you.

I certify that an Examination Committee met on 9th September 2004 to conduct the final examination of Bimo Ario Tejo on his Doctor of Philosophy thesis entitled "Reductive Alkylation of *Candida rugosa* Lipase: Structural Approaches" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree.
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This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirements for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

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BPTI	bovine pancreatic trypsin inhibitor
CD	circular dichroism
CRL	*Candida rugosa* lipase
DHFR	dihydrofolate reductase
GCL	*Geothricum candidum* lipase
HF	Hartree-Fock
MD	molecular dynamics
NMR	nuclear magnetic resonance
PDB	Protein Data Bank
PEG	polyethylene glycol
PME	Particle Mesh Ewald
RESP	restrained electrostatic potential
rmsd	root mean square deviation(s)
SASA	solvent accessible surface area
TNBS	trinitrobenzenesulfonate